Amendments to the Specification

1) Please replace the paragraph beginning on page 87 at line 12 and ending on page 88 at line 2, with the following replacement paragraph:

Expression of recombinant, secreted ASP-2 in insect cells

Ac-ASP-2 (SEQ ID NO: 20) was secreted a concentration of approximately 2 mg.L -1 by S*f*9 cells into culture medium. The protein was purified using nickel-NTA agarose and resolved as two closely migrating bands of 24-25 kDa (not shown). Both bands were recognized by monoclonal antibodies raised to the vector-derived, C-terminal V5 and His epitopes (not shown). The five N-terminal amino acids were sequenced from both bands and they were identical: G-M-R-N-S (SEQ ID NO: 115) where G-M-R is derived from the restriction site in the cloning vector, and N-S are the first two amino acids of mature7 (processed) *Ac*-ASP-2 (SEQ ID NO: 20) . Mass spectroscopy revealed the molecular weight of the major peak to be 24,492.2 Da (Fig. 46); this is in agreement with the predicted molecular weight of the secreted fusion protein (25,439.9 Da) in the absence of glycosylation. *Ac*-ASP-2 (SEQ ID NO: 20) was predicted to contain one N-linked glycosylation site at Asn-204, and treatment of the recombinant protein with PNGaseF removed the majority of protein that resolved in the upper band (data not shown). *O*-glycosidase treatment did not have an effect on the apparent molecular weight of recombinant *Ac*-ASP- 2 (SEQ ID NO: 20). Rabbit antiserum raised to ASP-2 recognized the recombinant antigen as well as a protein of the expected size in L3 extracts from *N. americanus* (not shown), indicating that *N. americanus* L3 produce a protein with immunologic similarity to *Ac*-ASP-2.(SEQ ID NO: 20). A molecular model of *Ac*-ASP-2 (SEQ ID NO: 20) based on the known structure of a PRP family member (Ves v 5 from the yellow jacket) showed that the two sequences shared significant identity in fold, despite only 26% identity at the primary sequence level (not shown). ASP-2 retained the general α, β, α core sandwich fold displayed by PRPs 22 .

2) Please replace the paragraph beginning on page 105 at line 20 and ending on page 106 at line 20, with the following replacement paragraph:

Secretion of catalytically active, glycosylated *Ac*-CP-2 by *P. pastoris*

Ac-cp-2 cDNA (GenBank accession number U18912) was cloned and reported by Harrop and colleagues [6]. We expressed recombinant *A*c-CP-2 as a secreted fusion protein in *P. pastoris* with a yield of 35 $mg.L^{-1}$. Secretion was mediated by the α-mating factor signal peptide derived from the pPIC-Z α vector. The protein was purified from *P. pastoris* culture supernatant using nickel-agarose [20]. The purified protein migrated with an apparent molecular size of 48 kDa (not shown). This was higher than the predicted size of the pro-enzyme (41.8 kDa) and processed, mature enzyme (32.1 kDa) factoring in the C-terminal *myc* and His tags and –terminal EAEAEF (SEQ ID NO: 116) motifs (introduced by the choice of restriction sites used in cloning of the construct). N-linked glycosylation of the 5 predicted sites in *Ac*-CP-2 probably accounted for some of the discrepancy between the predicted and observed molecular sizes. Deglycosylation with P*N*GaseF reduced the apparent molecular mass of recombinant *A*c-CP-2 by 5-10 kDa although numerous bands within this size range were apparent (not shown), probably corresponding to partially deglycosylated proteins. N-terminal amino acid sequencing of the major secreted protein by Edman degradation showed the N-terminal residue to be Glu-13, suggesting that some post-translational processing of the pro-region had occurred. However, this did not correspond with the predicted cleavage site of the pro-region from the mature enzyme (Asp-81 - Asp-82 using the numbering scheme of the fusion protein presented here). Although this is only a predicted cleavage site based on the known cleavage site of the pro-region of other related enzymes [9], it is unlikely that Glu-13 is the N-terminal residue of the native, secreted protease. Difficulty in obtaining sufficient quantities of native, hookworm-derived *A*c-CP-2 precluded N-terminal sequence information for comparison. Nonetheless, numerous faint bands with molecular sizes ranging from 30-40 kDa appeared when the purified recombinant *Ac*-CP-2 was stained with silver (not shown), suggesting that a small quantity of the recombinant protein was correctly processed to yield the mature form of the enzyme. This was further confirmed by the catalytic activity seen when recombinant *Ac*-CP-2 was incubated with Z-Phe-Arg-AMC (Figure 51). A broad pH range was observed with activity detected between pH 4-8 with optimal catalysis between pH 5 and pH 7. Addition of the cysteine protease inhibitor, E64, to a final concentration of 5 μM completely ablated cleavage of the peptide substrate (not shown).

Moreover, other recombinant proteins (non-proteolytic) expressed and purified in an identical fashion in our laboratory did not cleave Z-Phe-Arg-AMC (not shown).

3) Please replace the paragraph beginning on page 123 at line 9 and ending on page 123 at line 20, with the following replacement paragraph:

Based on the sequence obtained, forward and reverse primers were selected (both with and without histag, and all with EAEAEF (SEQ ID NO: 116) vector sequence) and synthesized (Integrated DNA Technologies, Inc., Coralville, IA). These primers were used to amplify *Na-asp-2* cDNA from the 1st strand Na-L3 cDNA. Na-L3 cDNA from mRNA extracted from L3 as described previously (Zhan et al, 2000). The L3 were obtained from golden hamsters infected with N. americanus as described previously (Xue et al, 2003). The PCR products were ligated into pPICZ A using EcoR1 and Xba1 sites. The ligation product was transformed into *E. coli* DH5 competent-rendered cells and the recombinants were selected by growing on LB-Zeocin plates. Eight colonies were picked from each transformation (with and without histag) and analyzed by PCR with vector primers. Each of the positive clones contained an insert of the predicted size. From two clones (one with histag and the other without) the plasmid was extracted and sent for DNA sequencing (Nevada Genomics Center).